Gene Therapy for Rare Diseases
(muscular dystrophies)

NATIONWIDE CHILDREN'S
When your child needs a hospital, everything matters.℠

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Harper Lab Program Goals:

Develop gene therapy approaches for dominantly inherited muscular dystrophies

- RNA interference (RNAi)

Rare Diseases

- NIH: diseases affecting less than 200,000 people in U.S.
- INSERM: 1 in 2,000 Europeans
- >7,000 rare disorders known; not all are genetic diseases[1,2]
- Affect 18 - 25 million Americans (6-8% of population)[1]
- Maybe affect 300 million people worldwide[3]
- FY2011: ~9,400 research projects on rare diseases ($3.5B / 11% of NIH research budget)[4]

[1]NIH Office of Rare Diseases Research
[2]Orphanet, INSERM
[3]Globalgenes.org/rarelist
[4]report.nih.gov/rcdc/categories

All myopathies are rare disorders

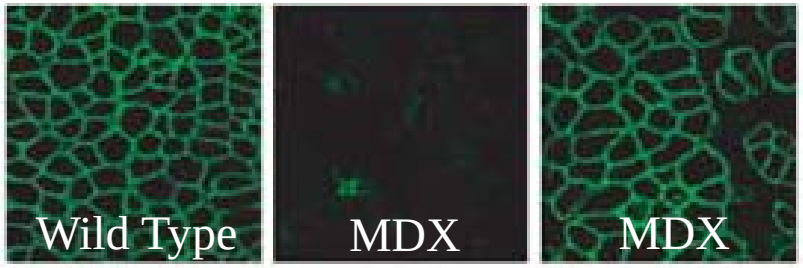
- Myopathy = muscle disease
- The Muscular Dystrophy Association (MDA) website lists 91 categories of muscle disease – some overlap between categories[1]
- NINDS groups myopathies into 12 subclasses[2]
- The **Muscular Dystrophies** are a group of disorders representing one major subclass of myopathy
- Dominant and recessive forms
- ~50 genes involved in various forms of muscular dystrophy

[1]www.mda.org

[2]www.ninds.nih.gov/disorders/myopathy/myopathy.htm

Muscle Gene Therapy

- Emerged from the identification of **Dystrophin** mutations as the cause of X-linked recessive Duchenne Muscular Dystrophy in 1986
  - DMD is the most common muscular dystrophy (1 in 3,500 newborn males)
  - ~8,000 males in U.S. extrapolates to 1 in 19,000 relative to total U.S. males
  - First positionally cloned muscular dystrophy disease gene
- **Replacing** missing or non-functional *dystrophin* seemed a straightforward DMD treatment (tools were available for gene replacement)



Green=DYS | No DYS | DYS gene replacement

Dystrophin gene replacement in mdx mouse model of DMD;
Gregorevic, et al Nat Med 2006

Historically DMD has been a dominant focus for the muscle gene therapy field

FY2011: 43% of "myopathy/MD" grants on DMD

$3.6B

NIH expenditures on rare diseases in FY2011

$75M

- NIH grants on "myopathy/muscular dystrophy" FY2011
- 0.15% of rare disease budget

$32M

- NIH grants on "DMD" FY2011
- 43% of $75M

"Follow the money"

Source: report.nih.gov/categorical_spending.aspx

FY2011: 85% of muscle gene therapy grants on DMD

$248M

- NIH grants on "gene therapy" FY2011

$5.6M

- NIH grants on "gene therapy for myopathy/muscular dystrophy" 2.2% of NIH gene therapy grants

$4.8M

- NIH grants related to "DMD gene therapy"

DMD *gene replacement* focus facilitated muscle gene therapy research in general

- Advancements in DMD gene therapy are applicable to other muscular dystrophies
  - Recessive diseases: Successful Phase I α-sarcoglycan gene replacement trial for **LGMD2D** (Mendell, JR et al Ann of Neurol 2008 and 2010)
  - Dominant diseases: UNEXPLORED UNTIL NOW - My lab's focus

Emergence of RNAi provided the tools to treat dominant myopathies with gene therapy

Dominant Myopathies

- At least 37 different loci involved in dominant myopathy
- All rare to extremely rare
- Silencing of mutant allele may be a common therapeutic strategy
- Collectively prevalence unclear but may be 1 in 2,400 to 1 in 3,200
  - Could affect ~130,000 people in U.S.

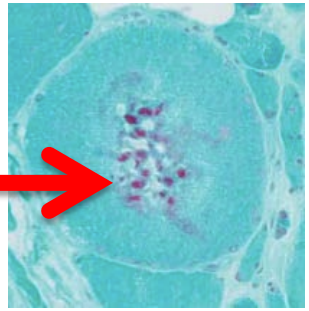
- My lab is developing therapies for two dominant MDs:

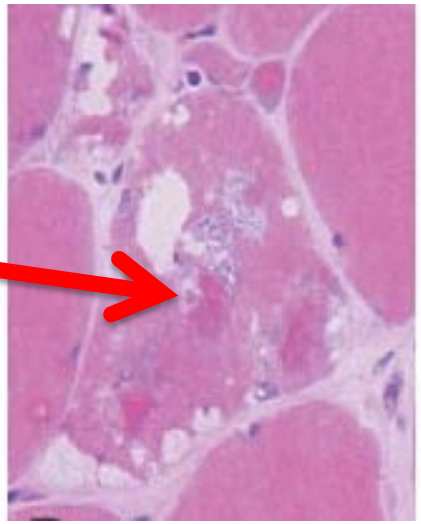
FSHD	**LGMD1A** ←
1 in 7,500 – 20,000	~ 1 in 1,000,000
16K – 42K Americans	~315 Americans
Most common dominant MD?	Good paradigm for first translation

LGMD1A

- Age of onset: 18-37 years
- Develop proximal leg and arm weakness in early adulthood and progress to the distal limb
- Myopathic features: variable fiber size, central nuclei, mononuclear infiltration
- Eosinophilic protein aggregates
- Gain-of-function mutation in the Myotilin (MYOT) gene

Myotilin-positive protein aggregates

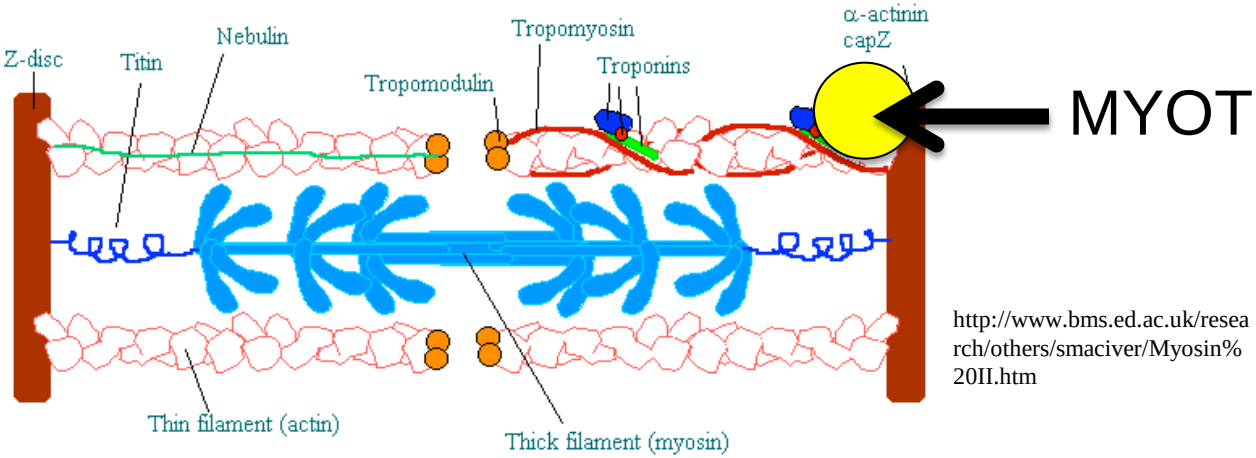




Olivé M et al. Myotilinopathy: refining the clinical and myopathological phenotype. Brain. 2005;128:2315-26.

Myotilin

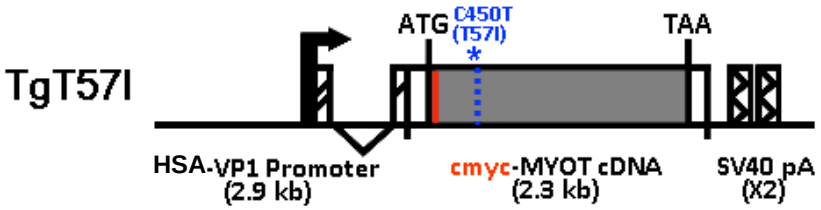
- Expressed primarily in skeletal and cardiac muscle
- Functions as a structural component of the Z-disc



http://www.bms.ed.ac.uk/research/others/smaciver/Myosin%20II.htm

- **Not required for normal muscle development or function**
- Mouse and human myotilin transcripts have the same expression pattern and are highly conserved

Myotilin T57I Transgenic Mouse Model

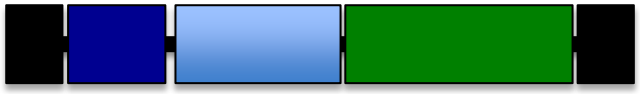


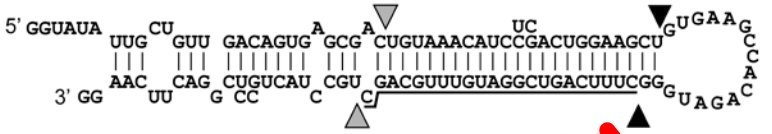
Garvey S M et al. Hum. Mol. Genet. 2006;15:2348-2362

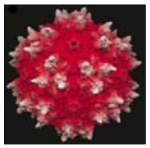
Hypothesis: Reducing the levels of mutant MYOT will offer a treatment for LGMD1A

Strategy: Develop RNAi gene therapy to suppress mutant MYOT

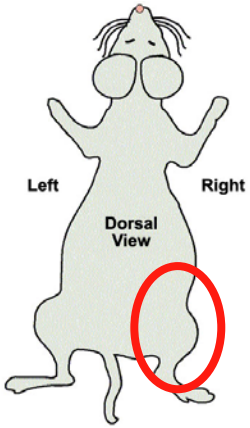
Strategy: Deliver MYOT-targeted miRNAs to LGMD1A mice

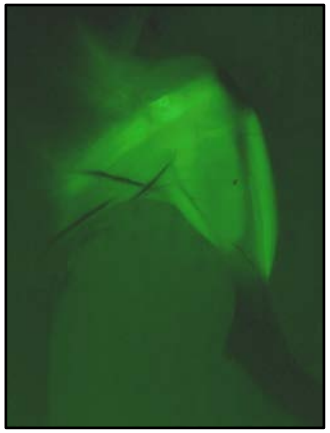




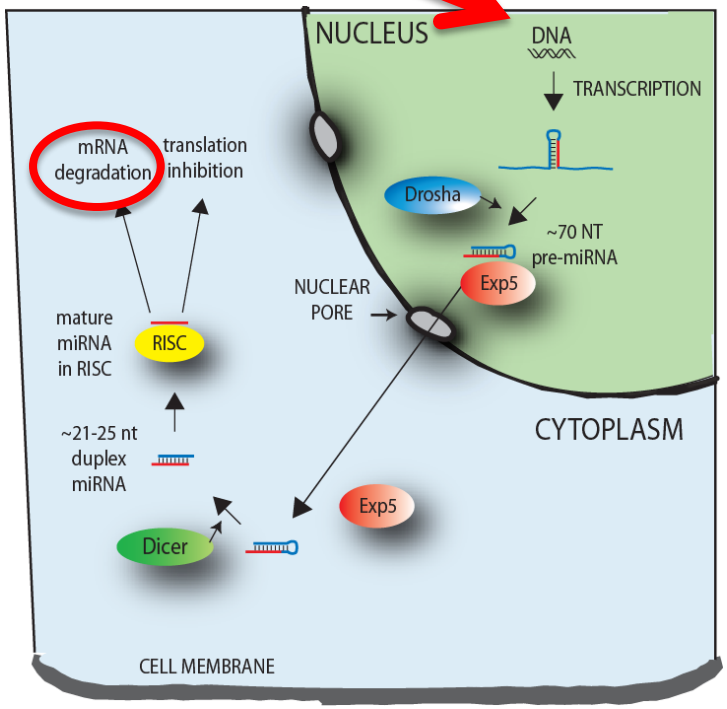


AAV6

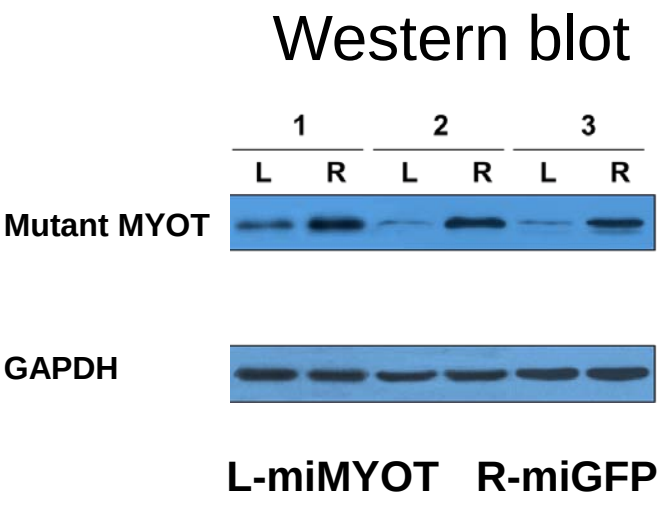


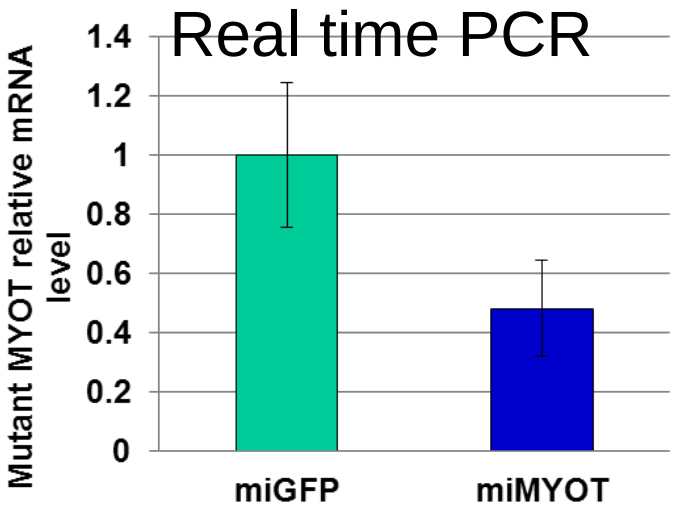


MYOT Knockdown

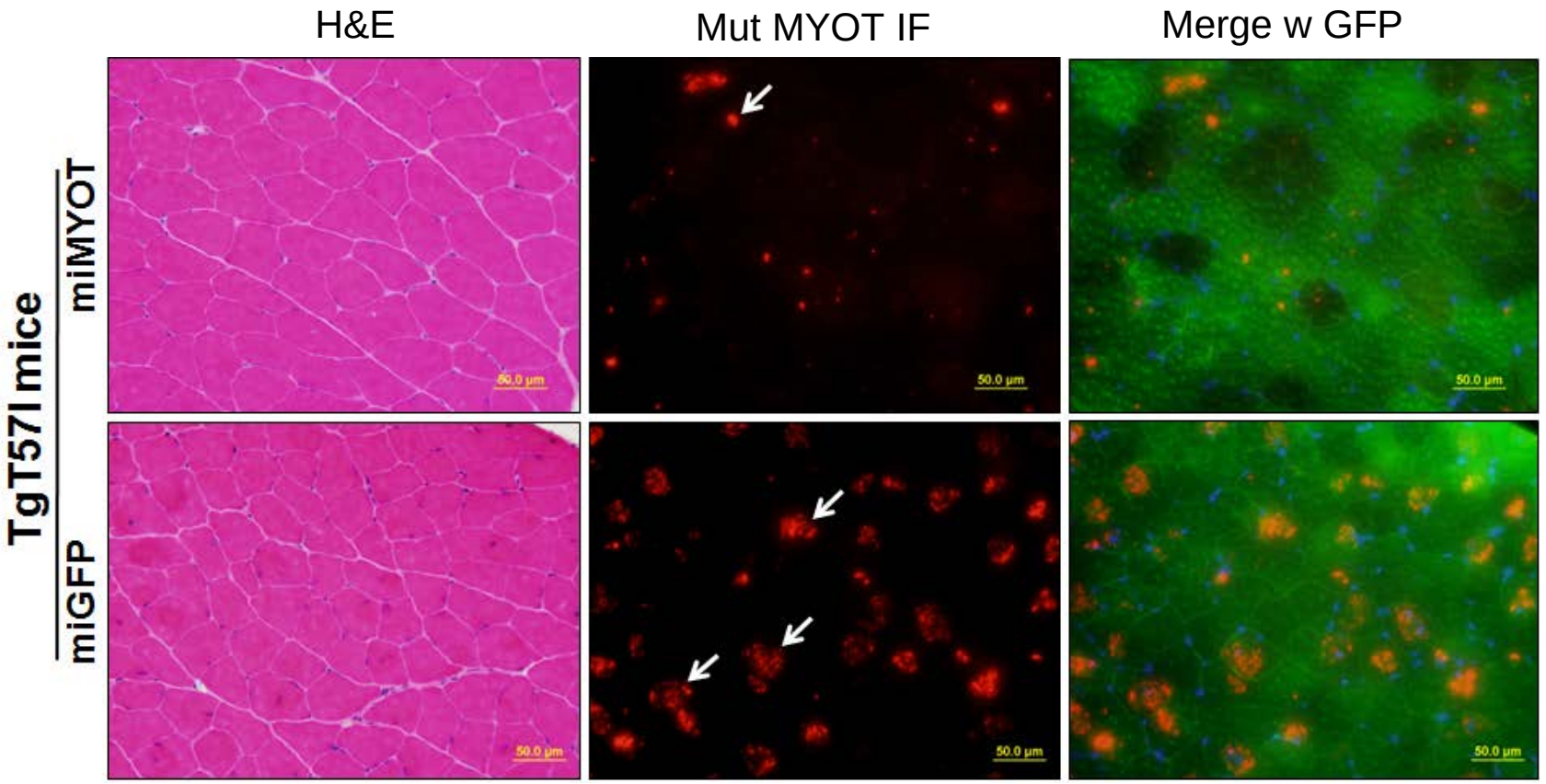


AAV.miMYOT mediates efficient MYOT knockdown *in vivo*



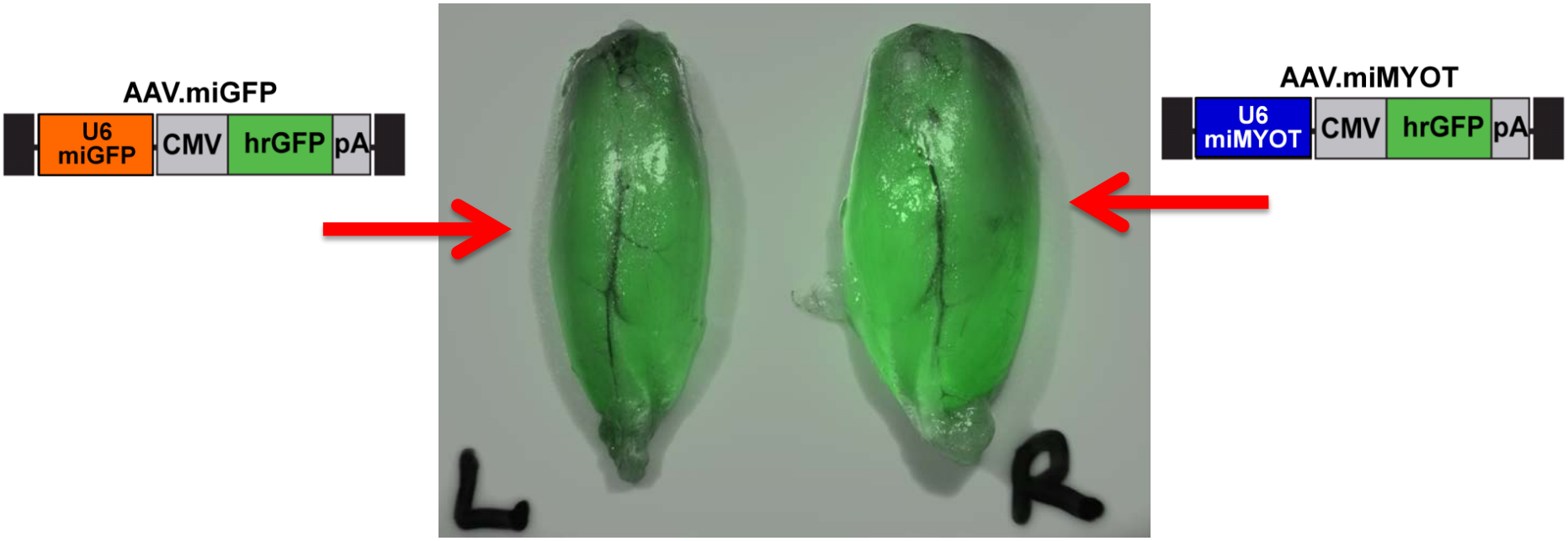


miMYOT Silencing Reduces Protein Aggregates by 70%



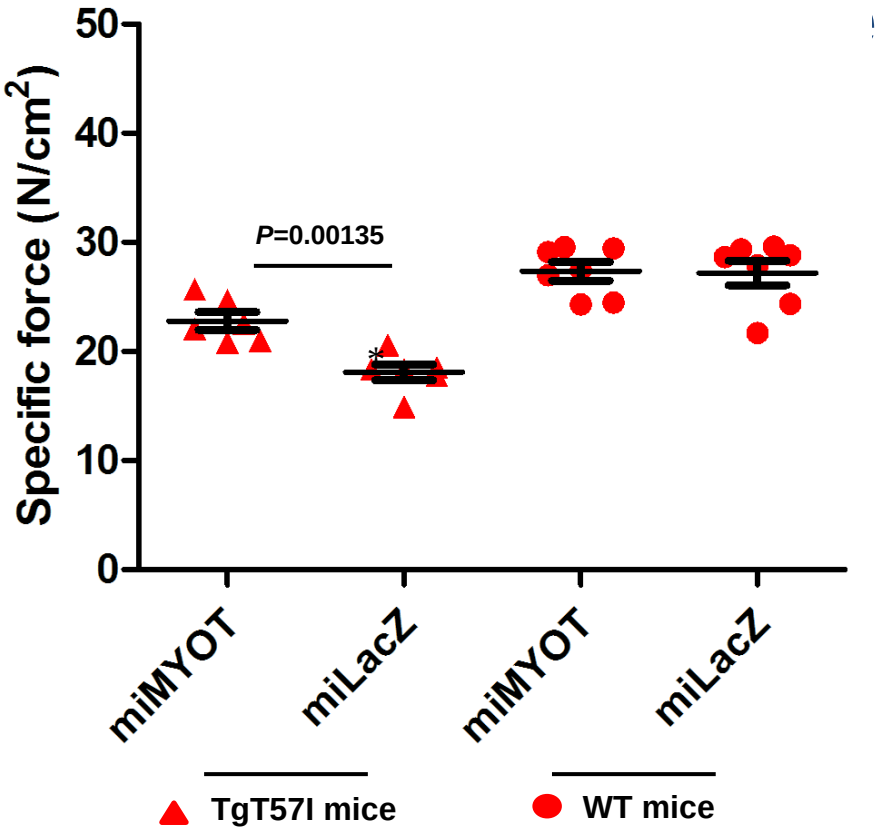
MYOT Silencing Improves Muscle Mass in 3 and 9-month old mice

MYOT T57I gastroc muscles



Overall ~ 10% increase in muscle mass; N=12 mice; p=0.0019

MYOT silencing improves whole muscle strength



Overall ~ 40% improvement

Conclusions

- MYOT gene silencing improves muscle abnormalities in mice over-expressing human mutant MYOT
- Moderate expression of miMYOT slows disease progression
- RNAi-based approaches may provide a therapy for LGMD1A and other dominantly inherited myopathies

Ongoing Work

- Increased dosage of miMYOT may further improve correction (remove GFP)
- Reversal study ongoing
- Preliminary tox studies ongoing
- IP filed

Hurdles to translation

- Immunology issues
- Need good natural history studies
  - "clinically relevant endpoints"
  - Gene expression/knockdown is not enough – must produce functional improvements
  - Biomarkers must correlate to some functional correction
- Navigating the patth forward from pre-clinical to IND is confusing and daunting
  - What are the steps required? Which academic scientists can advise on how to write an IND (~600 pages!)? What's involved in pre-pre-IND, pre-IND meetings with FDA? How do we fund IND-enabling tox, which can run ~$300K? Where do we produce vector? How do we fund it?
- Vector Production – what do we need for systemic muscle gene therapy? 10^{16} particles per person?
  - 1000 liter prep = ~10^{17} = 10 patients: probably max capacity of academic GMP vector core
  - Estimated $500k - $1M for one prep
  - Contract manufacturing needed to provide economy of scale

Acknowledgements

- Harper Lab – Nationwide Children's Hospital
  - Dr. Jian Liu
  - Dr. Lindsay Wallace, Sara Garwick-Coppens

- Collaborators:
  - Jerry Mendell (Nationwide Children's Hospital)
  - Michael Hauser (Duke University)
  - Susan Brooks (University of Michigan)
  - Chady Hakim (University of Missouri)

  Other expertise at NCH: Reed Clark, Kevin Flanigan, Chris Schilling, Jerry Mendell

- Funding for LGMD1A pre-clinical work
  - NINDS 1R21NS072260-01
  - Nationwide Children's Hospital

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Gene replacement is not useful for dominantly inherited disorders